

AD _____

Award Number: DAMD17-98-1-8160

TITLE: Examination of the Role of Membrane Type-1 Matrix
Metalloproteinase in Breast Cancer Metastasis

PRINCIPAL INVESTIGATOR: Jian Cao, M.D.
Stanley Zucker, M.D.

CONTRACTING ORGANIZATION: State University of New York
Stony Brook, New York 11794-3366

REPORT DATE: August 2000

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20010511 170

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE August 2000	3. REPORT TYPE AND DATES COVERED Annual Summary (1 Aug 99 - 31 Jul 00)	
4. TITLE AND SUBTITLE Examination of the Role of Membrane Type-1 Matrix Metalloproteinase in Breast Cancer Metastasis		5. FUNDING NUMBERS DAMD17-98-1-8160	
6. AUTHOR(S) Jian Cao, M.D. Stanley Zucker, M.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) State University of New York Stony Brook, New York 11794-3366 E-MAIL: jcao@mail.som.sunysb.edu		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words)			
14. SUBJECT TERMS Breast Cancer		15. NUMBER OF PAGES 7	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Report.....	4

TECHNICAL RESEARCH PROGRESS REPORT

Principal Investigator: Jian Cao, M.D.
Mentor: Stanley Zucker, M.D.
Grant Number: DAMD17-98-1-8160
Project Title: Examination of the role of membrane type-1 matrix metalloproteinase (MT1-MMP) in breast cancer metastasis

Recent studies have demonstrated that the production of tissue degrading enzymes (proteinases) by cancer cells lead to cancer dissemination. Matrix metalloproteinases (MMPs) are the major tissue degrading enzymes responsible for metastasis. Among these enzymes, membrane type matrix metalloproteinases are recently described MMP family members. In contrast to other MMPs, MT-MMPs are anchored in the plasma membrane and activate progelatinase A (also named MMP-2). Since gelatinase A is the MMP most commonly incriminated in breast cancer metastasis, the novel function of MT-MMPs appears important in breast cancer. Because of its central role in cell surface proteolysis, a more complete understanding of the role of MT-MMPs in cancer will expedite progress in therapy of metastasis. To this end, this project has been focused on the role of MT1-MMP in experimental breast cancer invasion and metastasis.

Task 1. Examination of the role of wild type membrane type-1 matrix metalloproteinase (MT1-MMP) in breast cancer metastasis

The study for the examination of the role of wild-type MT1-MMP in breast cancer metastasis has been done during the first two years as scheduled in this fellowship grant. The accomplishment for this section was reported to USAMR last year. In summary, a stable cell line expressing MT/GFP fusion protein was established in a human breast cancer cell line, MDA-MB-436. The cells stably expressing MT/GFP resulted in progelatinase A activation. The localization of MT/GFP in cells was found primarily in the endoplasmic reticulum and in the perinuclear Golgi apparatus. Fluorescence on the leading edge of plasma membrane was also observed but seems limited. By immunofluorescent study using MT1-MMP antibody and TRITC conjugated secondary antibody, the cell surface of MT1-MMP were confirmed. These data indicated that MT1-MMP primarily accumulated in the perinuclear region of transfected cells and may require a signaling mechanism to

facilitate membrane trafficking; GFP does not change the distribution of fused protein. Using GFP as a tag molecule to trace the invasive ability of MDA-MB-436 stable expressing MT1-MMP, I found MT-MMP enhanced the invasive ability of breast cancer cells more than two-fold compared with vector transfected cells. Native MDA-MB-436 cells produce slow growing, poorly invasive tumors. Injection of MDA-MB-436 cells stably transfected with MT1-MMP/GFP cDNA into the inferior mammary fat pad of female nude mice resulted in enhancement of tumor growth and local metastasis as compared to GFP alone transfected tumor cells. Thus, MT1-MMP may trigger the invasion of breast cancer cells by activating progelatinase A and thereby enhance tumor metastasis.

Task 2. Determine whether soluble forms of MT1-MMP play a role in experiment breast cancer dissemination (months 18-30)

To examine whether soluble form of MT1-MMP play a role in experimental breast cancer dissemination, we made a soluble MT1-MMP lacking the C-terminal transmembrane domain and cytoplasmic tail fused with GFP cDNA as a fusion protein. Without the transmembrane domain of MT1-MMP, this chimera is able to secrete into culture medium and is no longer anchored on the plasma membrane examined by immunofluorescent assay. To directly investigate the substrate specificity of MTATM, we purified the soluble form of MT1-MMP by using a His tag. A cDNA encoding the His tag was fused to the C-terminus of mutant MT1-MMP lacking the transmembrane domain as a chimera MTATM/His. MTATM/His purified from transfected COS-1 cells was appeared as 63kDa and 57 kDa proteins in conditioned medium and only 57 kDa form of MTATM/His (active form of MT1-MMP) has enzymatic activity against gelatin examined by gelatin zymography. MTATM/His is also able to digest type I and type IV collagen by using commercially available fluorescent labeled substrates. It was also noted that purified MTATM/His can activate progelatinase A. Therefore, soluble form MT1-MMP appears important role in the degradation of the component of basement membrane. Currently, stable cell lines for both MTATM/GFP and MTATM/His are being selected for stable expression in MDA-MB-436 cells and CHO-K1 cells, respectively. The role of soluble form of MT1-MMP in breast cancer metastasis will be determined in Boyden chambers and in nude mice.

Task 3. Investigate the trafficking and localization of MT1-MMP in transfected breast cancer cells (months 25-36)

A) We have demonstrated that Con A induces rapid activation of progelatinase A and rapid appearance of MT1-MMP at the cell surface by gelatin zymogram and cell surface biotinylation techniques, respectively in HT1080 cells. By employing same approach along with immunohistochemistry, I will examine the trafficking of MT-MMP in human breast cancer cells, MDA-MB-436. As initially planned, their task will be performed in year 3.

B) Pro-sequence of MT1-MMP serves as an intramolecular chaperone for the production of active MT1-MMP in transfected COS-1 cells.

The goal of this study is to further clarify the role of the propeptide domain of MT1-MMP trafficking to the plasma membrane and in maintaining the function of the plasma membrane-inserted enzyme. We propose that the propeptide sequence of MT1-MMP serves as an intramolecular chaperone in protein folding. To explore the concept, we have co-transfected COS-1 cells with the plasmids encoding the N-terminal propeptide domain of MT1-MMP and the MT1-MMP cDNA lacking the entire propeptide sequence and have demonstrated reconstitution of function of MT1-MMP. Co-transfection of COS-1 cells with both expression vectors resulted in reconstitution of MT1-MMP function in terms of facilitating ¹²⁵I labeled TIMP-2 binding to transfected COS-1 cells and subsequent activation of progelatinase A. Transfection of cells with either cDNA alone or cDNA for the propeptide of collagenase-1 with MTΔpro resulted in non-functional cells. Smaller cDNA mutations of the open reading frame of the N-terminal propeptide of MT1-MMP were employed to delineate critical conserved regions of the molecule required as a membrane-bound enzyme. These data indicated that the MT1-MMP prosequence acts as an intramolecular chaperone and is necessary for the correct folding of the MT1-MMP in vivo.

Task 4. This postdoctoral fellowship grant provides me with important experience of studying experimental animal models of breast cancer and in evaluating the effect of mutating MT1-MMP cDNA on the frequency of experimental metastasis. More importantly, Dr. Zucker meets me for 2 and a half hours per week to discuss experimental plans and to analyze

the results from completed experiments. This experience gave me the opportunity to learn how to become an independent investigator using molecular biology techniques to study the role of MMPs in tumor invasion and metastasis.

Relevant publication directly supported by this fellowship grant:

Cao, J., Hymowitz, M., Conner, C., Bahou, W., Zucker, S.
The propeptide domain of membrane type-1 matrix metalloproteinase acts as an intramolecular chaperone when expressed in trans with the mature sequence in COS-1 cells. J. Biol. Chem. (in press) October, 2000